# Topoisomerase II-alpha Gene Amplification as a Predictor of Responsiveness to Anthracycline-Containing Chemotherapy in the Breast Cancer International Research Group 006 Clinical Trial of Herceptin (Trastuzumab) in the Adjuvant Setting

Press MF, Sauter G, Buyse M, Bernstein L, Guzman R, Santiago A, Ma Y, Eiermann W, Pienkowski T, Crown J, Robert N, Bee V, Taupin H, Villalobos I, Seelig S, Pegram M, and Slamon DJ.

University of Southern California, Los Angeles, CA; University of Hamburg, Germany; IDDI, Brussels; GBG, Munchen, Germany; Maria Sklodowska-Curie (MSC) Centre, Warsaw, Poland; ICORG, Dublin, Ireland; US Oncology, Dallas, TX; BCIRG, Paris, France; Abbott-Vysis, Inc., Downers Grove, IL; U.C.L.A., Los Angeles, CA

## Abstract

**Background.** TOP2A gene amplification, not HER-2 amplification, may be the predictive marker for responsiveness to anthracycline chemotherapy because approximately 40% of breast cancers with HER2 gene amplification are also TOP2A gene amplified and because topoisomerase II-alpha is the known target for anthracyclines.

**Methods.** We evaluated topoisomerase II-alpha amplification by fluorescence *in situ* hybridization (FISH) in breast cancer tissue from women entered in the Breast Cancer International Research Group (BCIRG) 006 randomized clinical trial of trastuzumab (Herceptin®) in the adjuvant setting. The HER-2 gene amplification status was prospectively determined by FISH in one of two centralized laboratories prior to patient randomization. All women entered in the BCIRG006 clinical trial had breast cancers with HER-2 gene amplification. Two of the BCIRG006 treatment arms were anthracycline-containing (AC ->T and AC -> TH) and accrued 1074 patients in each treatment arm. We assessed the cumulative survival among the subgroups of patients defined by treatment with or without trastuzumab and TOP2A status.

**Results.** TOP2A status was determined by FISH in breast cancer tissue sections from 2120 of the 3222 women entered in the BCIRG006 clinical trial. Overall 744 (35.1%) breast cancers showed TOP2A gene amplification, 1285 (60.6%) showed a normal TOP2A ratio and 91 (4.3%) showed deletion of TOP2A. Women whose breast cancers showed TOP2A gene amplification had a significantly longer disease-free survival than women whose breast cancers had either normal TOP2A or deleted TOP2A. Women whose breast cancers lacked TOP2A gene amplification and were treated with AC -> T (arm 1) had a significantly shorter disease-free survival than women who were treated in either trastuzumab (Herceptin)-containing treatment arm (AC->TH or TCH). However, among women whose breast cancers had TOP2A gene amplification there were no significant differences in disease-free survival among the three treatment arms. Among the treatment arms a significant difference was also observed for overall survival with women whose breast cancers had TOP2A gene amplification showing a more favorable outcome. Women whose breast cancers lacked TOP2A gene amplification and were treated with AC->T (arm 1) had a significantly worse overall survival than women whose breast cancers lacked TOP2A gene amplification and were entered in either trastuzumab-containing treatment arm (AC->TH or TCH). However, among women whose breast cancers had co-amplification of TOP2A gene (with HER2 gene amplification) there were no significant differences in survival among the three treatment arms.

**Conclusions.** TOP2A gene amplification in breast tumors of women with HER-2 amplified breast cancers are associated with increased responsiveness to anthracycline-containing chemotherapy as indicated by a more favorable disease-free and overall survival. Co-amplification of TOP2A should be evaluated in breast cancers with HER2 gene amplification as a potential therapeutic target.

## Background

The human epidermal growth factor receptor type 2 (HER-2) gene encodes a membrane receptor protein in the epidermal growth factor receptor family. This gene is located on the long arm of chromosome 17 (17q11.2-12) not far from the locus for the topoisomerase II-aalpha (TOP2A) gene (17q21-22). Several studies have reported an association between HER2 gene amplification / overexpression and increased responsiveness to anthracycline chemotherapy; however, a biological mechanism to explain the association between HER-2 amplification and altered sensitivity to anthracyclines is not known. Experiments with mouse and human cells indicate that *in vitro*-induced HER-2 protein overexpression does not alter the chemosensitivity of cancer cells to anthracyclines. HER-2 protein, a transmembrane growth factor receptor, does not interact physically with topoisomerase II inhibitors. Although the HER-2 oncogene is considered to be the target gene for chromosome 17q12-q21 amplification, the amplicon may contain other closely associated genes, including v-erbA/thyroid hormone receptor (THRA); retinoic acid receptor; MLNs (metastatic axillary lymph nodes) 50, 51, 62, and 64; and topoisomerase II-alpha (TOP2A). Since the gene for topoisomerase II-$\alpha$, a well-established molecular target for anthracyclines in experimental systems, is frequently co-amplified with HER-2 in breast cancers because of their proximity on chromosome 17q, it is plausible, although unproven, that this may be the link between HER-2 overexpression and anthracycline responsiveness. In order to test this hypothesis we retrospectively evaluated topoisomerase IIα gene amplification in breast cancer tissue sections from a cohort of women with HER2-amplified breast cancer who were subjects in a randomized phase III international clinical trial of chemotherapy and trastuzumab (Herceptin®) in the adjuvant setting, the BCIRG006 clinical trial.

## Objectives

1. Assess the frequency of TOP2A gene amplification in the BCIRG 006 clinical trial.
2. Determine if TOP2A gene amplification status was associated with responsiveness to anthracycline-containing or trastuzumab-containing adjuvant therapy.

## Patients

The Breast Cancer International Research Group 006 study was a randomized phase III multinational trial of trastuzumab (Herceptin®) in the adjuvant setting. The patients were enrolled between March, 2001 and March, 2004. The study enrolled a total of 3222 patients, with 1073 patients randomized to receive "standard" breast cancer chemotherapy (AC->T) (treatment arm 1), 1074 randomized to receive "standard" chemotherapy with trastuzumab (AC-TH) (treatment arm 2) and 1075 patients randomized to receive chemotherapy agents that had been shown to interact with trastuzumab in a synergistic fashion with little known cardiac toxicity (TCH) (treatment arm 3). For enrollment in this trial, documentation of HER-2 gene amplification determined by fluorescence *in situ* hybridization (FISH) was required at one of two centralized laboratories (University of Southern California or University of Basel). Accrual to BCIRG006 was completed in March, 2004. The patients were consented at initial entry to the BCIRG 006 clinical trial and retrospective analysis of archival tissue sections from these patients' breast cancers for TOP2A gene amplification was approved by the USC Institutional Research Board.

In brief, patients with node-positive or high-risk, node-negative primary breast cancer were randomized to one of the three treatment arms. Arm 1 (control arm) consisted of four cycles of doxorubicin 60 mg/m$^2$ plus cyclophosphamide 600 mg/m$^2$ every three weeks followed by four cycles of docetaxel 100 mg/m$^2$ every three weeks (AC->T). Women in treatment arm 2 received the same AC followed by T chemotherapy treatment but, in addition, received trastuzumab immunotherapy (2mg/kg/week during chemotherapy and then 6mg/kg/week until 1 year of treatment) for one year. In treatment arm 3 (TCH) women received docetaxel 75 mg/m$^2$ with cisplatin 75 mg/m$^2$ or carboplatin at an area under the curve of 6 every three weeks for six cycles (with the choice of cisplatin or carboplatin determined by the institutional preference) and trastuzumab (2 mg/kg/week) for one year. Disease-free survival is the primary end-point for the study. Secondary endpoints include overall survival, distant relapse-free survival, safety and cardiotoxicity.

## Materials and Methods

Tissue Specimens. Paraffin-embedded tissue blocks or unstained paraffin-embedded breast cancer tissue sections, 4 to 6 μM thick, from potential subjects for the BCIRG006 study were submitted to either the USC or University of Basel BCIRG Central Laboratory for analysis of HER-2 status by FISH prior to entry in the clinical trial. All 3222 women except two who entered the BCIRG006 clinical trial have breast cancers with HER-2 gene amplification demonstrated by FISH. Tissue microarrays were prepared from those breast cancer specimens submitted as paraffin-embedded tissue blocks. Tissue sections from these tissue microarrays were used to assess topoisomerase II-alpha gene amplification as described below.

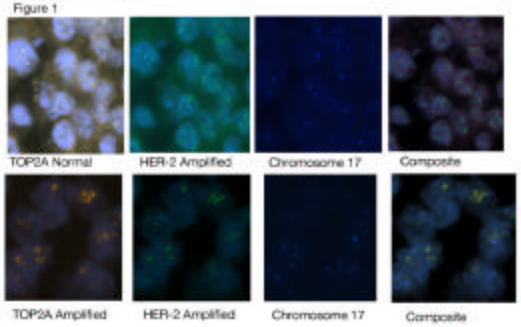
TOP2A and HER-2 Gene Amplification by Fluorescence *in Situ* Hybridization. Topoisomerase II-alpha and HER-2 gene amplification was determined by fluorescence *in situ* hybridization (FISH) using commercially available probes. We used the same FISH assay method as described elsewhere in detail for HER-2 gene amplification (Mass et al., 2005[1]). By using an LSI HER-2 probe labeled with SpectrumGreen (Vysis, Inc.) and an LSI TOP2A probe labeled with SpectrumOrange (Vysis, Inc.) simultaneous analysis of HER-2 and TOP2A were performed with the FISH assay. The number of chromosome 17 centromeres was determined by FISH using a CEP 17 probe labeled with SpectrumAqua (Vysis, Inc.). The number of TOP2A and HER-2 gene copy signals were determined in 20 interphase nuclei and compared with the number of chromosome 17 centromeres in the same interphase nuclei. HER-2 gene amplification as well as TOP2A gene amplification by FISH was defined as a HER-2 or TOP2A gene-to-chromosome 17 centromere ratio of $\geq$ 2.0. Our reasons for using FISH ratio $\geq$ 2.0 as the cut-off for gene amplification are 1.) The established "cut-off" used for evaluating gene amplification with Southern hybridization is a ratio of an index gene-to-control gene of 2.0 or greater; 2.) the accepted FDA-approved FISH ratio for HER-2 gene amplification is $\geq$2.0; 3.) because only a portion of a cell population is dividing at any one time, using a ratio of 2.0 or greater is not likely to lead to confusion with non-amplified, actively dividing cell populations. We have selected a FISH ratio of $\leq$0.8 as indicative of TOP2A gene deletion because we wish to identify breast cancers that lose a single gene copy from a tetraploid or near-tetraploid, aneuploid breast cancer (approximately 75% of breast cancers are either tetraploid or near-tetraploid). In addition, this is the ratio that has been used by the majority of investigators.

[1]Mass RD, Press MF, Anderson S, Cobleigh MA, Vogel CL, Dybdal N, Leiberman G, Slamon DJ. Evaluation of clinical outcomes according to HER2 detection by fluorescence *in situ* hybridization in women with metastatic breast cancer treated with trastuzumab. Clinical Breast Cancer 6: 240-246, 2005.

## Statistics

Patients were classified according to the treatment arm they actually received. Disease-free survival was defined as the time between randomization and date of breast cancer relapse, second primary malignancy or death, whichever occurred first. Recurrence-free survival was the same as disease-free survival including only breast cancer relapse and death from breast cancer as an event. Overall survival was the time from randomization to date of death from any cause. Presented hazard ratios are based on Cox model including the patients of interest.

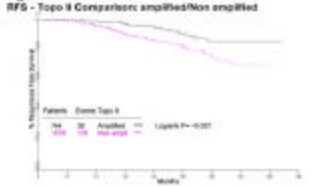
Figure 1




## Results

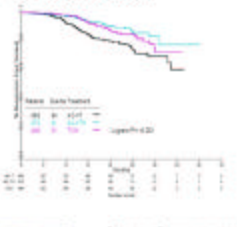
TOP2A gene status was determined by FISH (Figure 1) for 2120 of the 3222 (65.8%) women entered in the BCIRG006 clinical trial; 744 of the 2120 (35.1%) showed TOP2A gene amplification, 1285 (60.6%) were TOP2A normal and 91 (4.3%) were TOP2A deleted. Analysis of the disease-free and recurrence-free survival (RFS) by TOP2A status showed significantly longer DFS ($p < 0.001$) (data not shown) and RFS ($p < 0.001$) among women whose breast cancers had TOP2A gene amplification compared to those women whose breast cancers did NOT have TOP2A gene amplification (Figure 2). The differences in RFS between women whose breast cancers were TOP2A normal and TOP2A deleted were not statistically significant, so these cases were grouped together.

Figure 2



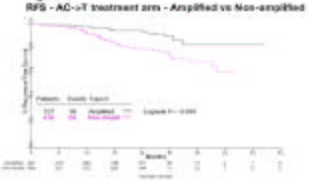

When analyzed by treatment arm there was a statistically significant difference in both DFS and RFS (Figure 3) for those women whose breast cancers LACKED TOP2A gene amplification (but had HER2 gene amplification). Those women whose breast cancers LACKED TOP2A gene amplification and were randomized to standard chemotherapy (AC->T, arm 1) had a worse outcome (DFS, $p < 0.001$; RFS, $p < 0.001$) than those women randomized to either of the trastuzumab-containing treatment arms (arms 2 or 3) (Figure 3). Neither the DFS nor the RFS differed significantly between treatment arm 2 and treatment arm 3 for women whose breast cancers LACKED TOP2A gene amplification (Figure 3). No significant differences were observed among the 3 treatment arms for those women whose breast cancers had co-amplification of both TOP2A and HER-2 genes (data not shown).

Figure 3



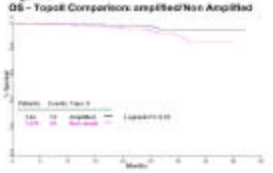

Among all women randomized to standard chemotherapy alone (arm 1, AC->T), those women whose breast cancers had co-amplification of TOP2A and HER-2 genes showed a significantly longer DFS ($p = 0.00158$) and RFS ($p < 0.001$) (Figure 4) compared to those women whose breast cancers LACKED TOP2A gene amplification (but had HER2 gene amplification). Women who were randomized to the AC->TH trastuzumab-containing treatment arm also showed a significantly longer RFS ($p = 0.019$) for those women whose breast cancers had TOP2A gene amplification compared to those women whose breast cancers lacked TOP2A gene amplification (data not shown).

Figure 4



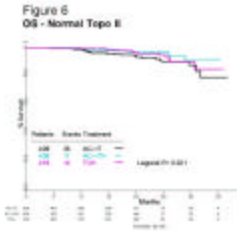

Overall survival was improved for women with co-amplification of TOP2A and HER-2 genes compared to overall survival of women with only amplification of HER2 ($p = 0.01$) (Figure 5). Overall survival of women with normal TOP2 gene copy number (and HER2 amplification) varied significantly by treatment arm.

Figure 5



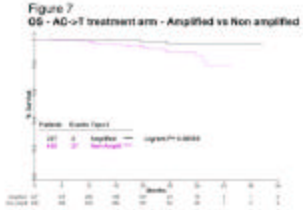

Those women with normal TOP2A gene copy number entered on the standard AC->T chemotherapy treatment arm had a significantly ($p = 0.021$) worse overall survival compared to women entered on either of the trastuzumab-containing treatment arms (AC->TH or TCH) (Figure 6). However, for women with normal TOP2A differences in overall survival between the two trastuzumab-containing treatment arms (AC->TH and TCH) were not significant (Figure 6). In contrast, overall survival of women with co-amplification of TOP2A and HER-2 genes was not significantly different among the three treatment arms (data not shown).

Figure 6




Among women randomized to the standard AC->T chemotherapy (arm 1) significant differences in overall survival were observed between women whose breast cancers had co-amplification of TOP2A and HER2 genes and those whose breast cancers LACKED TOP2A gene amplification but had HER2 amplification ($p = 0.00589$) (Figure 7). Among women randomized to the trastuzumab-containing treatment arms there was no statistically significant difference in the overall survival of women whose breast cancers had co-amplification of TOP2A and HER-2 genes compared to the overall survival of women whose breast cancers had only amplification of the HER-2 gene (data not shown).

Figure 7




## Conclusions

- **Amplification of the TOP2A gene was significantly correlated with responsiveness to adriamycin-containing chemotherapy and may, therefore, be considered a predictive marker.**
- **Among women whose breast cancers LACKED TOP2A gene amplification, the trastuzumab treatment arms showed a significant association with more favorable clinical outcomes.**
- **Among women whose breast cancers showed co-amplification of TOP2A and HER2 genes there was no significant correlation with clinical outcome among the three treatment arms at this time.**